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HEMOGLOBIN DETERMINATION OF 1525 ADULTS
IN ELLIS AND THE SURROUNDING COUNTIES OF KANSAS

being

A thesis presented to the Graduate Faculty
of Fort Hays Kansas State College in
partial fulfillment of the requirements for
the degree of Master of Science

by

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Date April 26, 1961

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Chairman Graduate Council

THESIS ABSTRACT

An investigation was made to determine the hemoglobin levels of a sample of an adult population. The problem was three-fold in purpose: (1) to determine the number of adults having a low hemoglobin, (2) to determine if there was a difference in the percentage of males and females who might have low hemoglobin, and (3) to determine whether hemoglobin screening could be carried out efficiently and effectively with the other tests being completed in the screening programs by the Kansas State Board of Health.

During October of 1959 capillary blood samples were taken from 1525 adults of Ellis and the surrounding counties for the purpose of determining hemoglobin by the cyanmethemoglobin method using a Leitz Colorimeter. Any value lower than 11 grams per cent for females, and 12 grams per cent for males, was reported as being positive. The screening level for the hemoglobin test was established and approved by the Ellis County Medical Association. A pertinent medical history was obtained, and noted on the registration form for each person screened.

The author screened 946 females and 579 males in the survey. The mean hemoglobin for the females was 12.2 grams per cent and the mean for the males was 13.6.

The hemoglobin screening survey showed a 15.3 per cent positive rate of the total number screened. Sixteen and three-tenth per cent of the total female population screened positive as compared with 13.6 per cent of the total males who screened positive.

There were only five males of the total 79 positive males who reported having a previous history of anemia, as opposed to 67 females

of the total 154 positive females who reported that they had a previous history of anemia.

A previous history of low hemoglobin was noted for 241 persons. There were 24 positive cases who were under medication at the time, and 72 cases with a previous history who screened positive during the survey. The number of positive tests with no history of a low hemoglobin was 161. This figure represents 69.1 per cent of the total positive cases.

The age intervals in which the survey showed that the males were most likely to exhibit a low hemoglobin are 69-72, 41-44, and 53-56. The age interval in which the female is most likely to show a low hemoglobin, according to the survey, is 37-40.

This study seems to indicate that there are a sufficient number of adults who have a low hemoglobin to warrant a mass screening for this condition; that there are almost as many males who suffer from this condition as there are females, although any females have been diagnosed during pregnancy; and that the cyanmethemoglobin method of hemoglobin determination, using the Leitz Colorimeter, can be efficiently and effectively carried out in a mass screening survey.

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Dr. Edwin P. Martin assisted the author in choosing a method to complete the study and offered advice concerning the problem, for which the writer is grateful.

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The author wishes to acknowledge his appreciation to Hadley Memorial Hospital for providing the Leitz Colorimeter during the screening survey.

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INTRODUCTION

An interest regarding hemoglobin screening was first generated while the author was assisting the State Board of Health with a screening program in several Northwest Kansas counties. A large number of adults, at the time that blood specimens for diabetes screening were being drawn, stated that they had been told that they had anemia, or that they were presently suffering from anemia. These comments concerning anemia prompted the writer to plan a hemoglobin screening survey for an adult population in order to determine (1) the number of adults having a low hemoglobin, (2) if there was a difference in the percentage of males and females who might have low hemoglobin, and (3) whether hemoglobin screening could be carried out efficiently and effectively with the other tests being completed in the screening programs by the Kansas State Board of Health.

In October of 1959, hemoglobin screening tests were completed by the author on 1525 adults in Ellis and the surrounding counties. The cyanmethemoglobin method was employed in the determination of hemoglobin. Screening tests for diabetes, glaucoma, blood pressure, and vision were also completed at that time by the State Board of Health, the Department of Social Welfare, the Ellis County Medical Association and the Ellis County Home Demonstration Unit.

In order to carry out an efficient and effective multiple screening program, the following basic principles must be observed: (1) the screening tests should be simple to administer, (2) they should be easy to interpret, (3) they should be relatively inexpensive, and (4) they should require little time to perform. The cyanmethemoglobin method

chosen by the author meets all of these prerequisites.

There have been several multiple screening surveys completed in the United States in which the determination of hemoglobin was a portion of the program. The Greater Atlanta Survey, 1950, the San Francisco Survey, 1951, the Vernon (Los Angeles County) Survey, 1952, the Georgia Survey, 1954, and the Michigan Pilot Screening Survey, 1954, all screened for low hemoglobin using the copper sulphate method (American Medical Association, 1955). By using the copper sulphate method, which requires three or four drops of blood, a medicine dropper, and many bottles of copper sulphate of increasing density, it is possible for one to determine the specific gravity of the blood, and from it the hemoglobin content within 10 per cent.

The technique consists of letting drops of whole blood fall into a graded series of solutions of copper sulphate of known specific gravity and noting whether the drops rise or fall in the solutions. For accurate determination forty solutions would have to be used to cover the entire blood range, 1.035 to 1.075. The hemoglobin is then estimated by placing a straightedge across a chart for calculating percentages of hemoglobin from the gravity of whole blood.

The copper sulphate method was not used in this survey for the following reasons: the method depends upon an indirect estimate of hemoglobin, and it necessitates a rather complex calculation to determine accurately the hemoglobin level.

The cyanmethemoglobin method, using the Leitz Colorimeter, was chosen for the following reasons: the determination is accurate within 0.5 per cent (Kolmer, 1945); human error is reduced to a minimum, since a chart is

compiled from the standard curve giving the conversions from per cent transmittance to grams per cent of hemoglobin; the procedure is rapid; and there is a minimum of clinical equipment to clean and prepare for the method.

The Journal of the American Medical Association was surveyed for 1958, 1959, and for the first six issues of 1960 in order to obtain the then most recent medical information and investigations available on the subjects of hemoglobin and anemia.

A Study of Multiple Screening, a booklet compiled by the American Medical Association was consulted for the results of the most recent hemoglobin screening surveys in the United States.

The basic physiological conditions relating to low hemoglobin were taken from recent reference books.

The clinical procedures and methods were taken from the current references on laboratory techniques.

Medical follow-ups were not completed on the positive cases in this screening survey because of a lack of time to deal with this aspect of the problem.

ERYTHROCYTES, HEMOGLOBIN, AND ANEMIA

The erythrocyte is a biconcave disc made up of an envelope containing a stroma or semirigid network in the meshes of which the red coloring matter of the blood, or hemoglobin, is held. The transportation of oxygen from the lungs to the tissues through the body is the most important function of hemoglobin, since almost all of the oxygen is carried in loose combination with the hemoglobin of the blood (Memmler, 1959).

Taber (1954), has defined hemoglobin as an organic compound with a molecular weight of 68,000 and composed of heme and globin. Heme is the metal complex containing iron and imparts a red color to the hemoglobin. Globin is a colorless protein containing many amino acids. Carlson (1955), has estimated that the average amount of hemoglobin in 100 cubic centimeters of blood is 15.6 grams.

Judy and Price (1958), conducted hemoglobin level and red blood cell counts in 7,300 female cases and found the mean hemoglobin to be 12.55 grams per 100 cubic centimeters. The author's findings, in which the mean hemoglobin for the females was 12.2 grams per cent, compare favorably with the study by Judy and Price.

Seasonal variations have an effect upon the hemoglobin levels in both females and males. Cullen (1959), found, in a three year study, that the highest incidence of anemia in 6,094 pregnant women in Dublin, occurred during the months of June, July, August, and September. He also found that the rate of anemia was the lowest during the months of December, January, and February. Hervey and Gibson (1957), in their study

of blood donors in Los Angeles, found that the donors showed a higher incidence of low hemoglobin in the summer months. Almost twice as many blood donors in Los Angeles were disqualified for low hemoglobin levels in July as were disqualified in January.

Since the author's hemoglobin survey was completed in October, this sample should represent an intermediate between the low hemoglobins expected in the summer and the higher hemoglobins normally found in the winter months.

Anemia was observed by Christie (1959), in all six members of an arctic expedition subjected to low environmental temperatures, moderately high altitudes, continuous daylight, and strenuous physical exercise for 3.5 months. There was a significant fall in mean hemoglobin levels from 12.9 to 10.8 within the first two weeks. Further hemoglobin studies completed in relation to these environmental factors would be of interest.

METHODS

The method used in determining hemoglobin of the 1525 adults in this study was the cyanmethemoglobin method and consists of the dilution of an accurately measured volume of blood in an accurately measured volume of solution that will convert hemoglobin to cyanmethemoglobin (Gradwohl, 1948; Kolmer, 1945; Todd, 1944). The optical density of this solution is then compared with that of the standard and the density is taken to be directly proportional to the concentration of the pigment.

The adults who attended the screening clinic were first registered indicating their name, age, sex, the name of their family physician, and a pertinent history if they had received treatment for anemia. They were conducted through the clinic where their blood-pressure was taken, their vision tested, and they were screened for glaucoma, after which two specimens of blood were taken (Fig. 1). The first was used in determining whether the adult had a high blood sugar as a check for diabetes, and the second was a 0.02 ml. sample of blood which was diluted in 5 ml. of the chemical solution for the determination of hemoglobin. The sample of blood and chemical diluent was mixed thoroughly and the pipette was rinsed three times with the diluent in the test tube. The blood and the diluent were mixed thoroughly by swirling the tube. The tube was allowed to stand for 10 minutes before a photometric measurement was completed. This procedure allowed cyanmethemoglobin to form.

The Leitz Colorimeter (Fig. 2), was calibrated at zero with a blank test tube; the blank was then removed and replaced with the tube con-

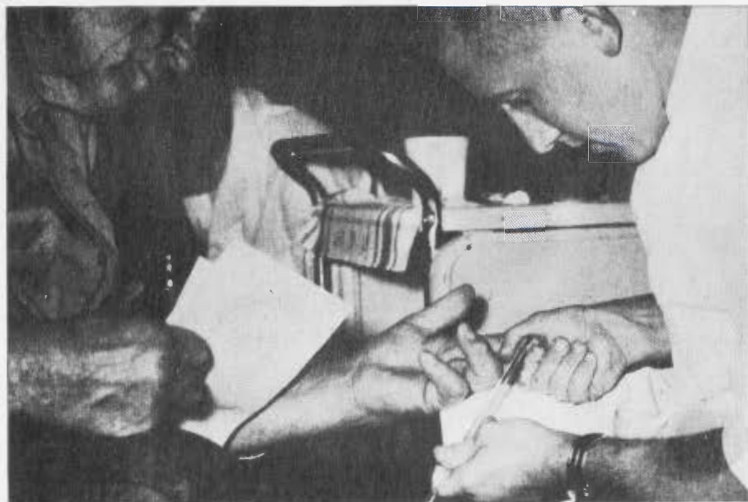


FIGURE 1

TECHNICIAN DRAWING A CAPILLARY BLOOD SAMPLE.

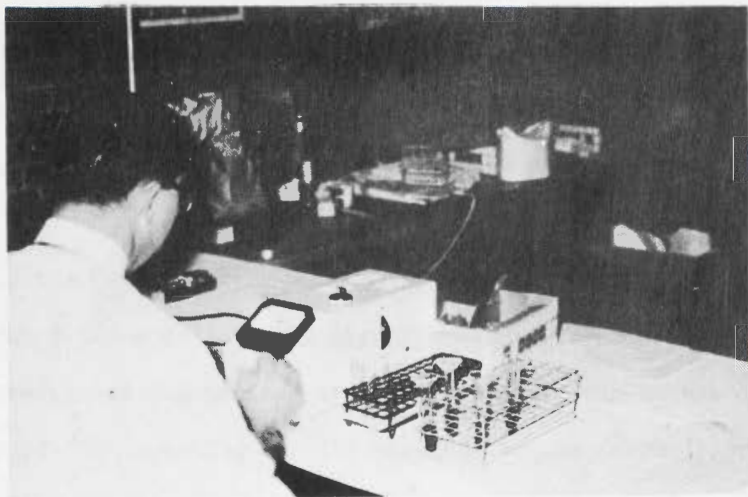


FIGURE 2

THE LEITZ COLORIMETER USED IN MEASURING THE DENSITY OF THE SOLUTION.

taining the specimen. The density reading was observed (Fig. 3), and the hemoglobin value equivalent to the density reading was observed from the standard curve and the figure was recorded on the registration form.

If the test resulted in a positive reading, below 11 grams per cent for females or below 12 grams per cent for males, the individual was interviewed and advised to visit his physician in the near future. A copy of the screening results was sent to the person's physician, along with a letter from the county health officer which explained the procedures of the screening survey.



FIGURE 3

THE AUTHOR READING THE DENSITY OF THE SOLUTION IN THE COLORIMETER.

STUDY AREA

The hemoglobin screening schedule in Ellis County included two days of operation in Hays and one day of operation in Ellis, 13 miles west of Hays. The clinic was operated in the National Guard Armory in Hays and at the Ellis High School Auditorium in Ellis.

Ellis County is located in the central northwest portion of Kansas and has a total population of approximately 21,000 persons. Hays, the county seat, has a population of 11,784, and the other two cities in the county are Ellis with a population of 2,311, and Victoria, which has a population of 1,159. The average altitude of Ellis County is around 2,000 feet. Not all of the adults screened in the survey were from Ellis County. There were 148 adults from the surrounding counties screened with the Ellis County residents. The majority of the adults tested were of German extraction, and both rural and city populations were represented in the hemoglobin survey.

There are 26 Doctors of Medicine practicing in Ellis County, and two hospitals are located in Hays, so that excellent general medical services are readily available to everyone in the county.

An adult population was chosen in this survey because opinions vary concerning the interpretation of the hemoglobin values obtained from blood samples drawn from children and adolescents.

RESULTS AND DISCUSSION

A total of 1525 blood specimens was taken from adults in Ellis and the surrounding counties of Kansas for the determination of hemoglobin levels. There were 946 females and 579 males screened in the survey.

The mean age of the total group was 48.6 years, while the mean age of the males was 49.0, and the mean age of the females was 48.3. The population was an unusual group in that one would not expect a mean age this high in a general population. However, Table I shows that there was a heavy concentration of cases in the age groups from 41 to 56. Another reason for the higher mean age is the fact that no cases were screened for a low hemoglobin under the age of 21. There were no earlier studies, concerning age groups, with which the investigator could compare his findings.

The mean hemoglobin for the females was 12.2 grams per cent and the mean for the males was 13.6. These means compare favorably with the mean hemoglobins given by Fulton (1949), and Scheer (1953).

Table I shows the distribution of cases by sex grouped into four year intervals. This table also shows the mean hemoglobin for each interval according to sex.

Column 3 (Table I), gives the deviation from the mean hemoglobin for the female and male. One trend of importance is the small variation between the mean hemoglobin of the females in age groups considering the means of each interval. However, as the deviation from the mean for each interval is compared with increasing age, it may be seen that the deviation remains approximately the same from 21-24 (-0.3), through age 52, and then in the following age interval, 53-56, increases to a plus 0.4. According

TABLE I

DISTRIBUTION, MEAN HEMOGLOBIN, AND DEVIATION FROM THE MALE
AND FEMALE MEAN ACCORDING TO AGE AND SEX *

AGE	NUMBER		MEAN HEMOGLOBIN		DEVIATION FROM THE MEAN HEMOGLOBIN	
	Male	Female	M	F	M	F
21-24	19	20	13.4	11.9	-0.2	-0.3
25-28	11	43	13.4	12.1	-0.2	+0.1
29-32	31	49	13.4	11.9	-0.2	-0.3
33-36	42	72	14.3	12.0	+0.7	-0.2
37-40	62	98	13.7	11.8	+0.1	-0.4
41-44	69	108	13.4	12.0	-0.2	-0.2
45-48	59	105	13.8	12.0	+0.2	-0.2
49-52	64	90	13.8	12.1	+0.2	-0.1
53-56	53	105	13.5	12.6	-0.1	+0.4
57-60	52	81	13.7	12.4	+0.1	+0.2
61-64	34	55	13.5	12.3	-0.1	+0.1
65-68	34	49	13.3	12.4	-0.3	+0.2
69-72	21	35	13.1	13.0	-0.5	+0.8
73-76	13	22	13.6	13.0	.0	+0.8
77-80	10	5	13.9	11.7	+0.3	-0.5
81-84	7	7	12.0	12.0	-1.6	-0.2
TOTAL	579	946				

* Note: This table is to be read in the following manner: there were 19 males in the age interval 21-24, their mean hemoglobin was 13.4, and the deviation from the total male's mean hemoglobin was -0.2.

to most authors the majority of women have passed menopause by the age of 52. It would be an interesting and informative investigation to complete a hemoglobin screening survey in a female adult population in which a record was kept of the females who had passed menopause. This study might show whether the women who had passed menopause had a higher mean hemoglobin than the women who had not passed menopause.

There was a total of 233 cases who screened below the hemoglobin values of 11 grams per cent for females and 12 grams per cent for males, and so were reported as positive. This figure represents a 15.3 per cent positive rate of the number screened.

These results compare favorably with a hemoglobin screening survey completed by the Richmond Virginia Department of Health, 1950, (American Medical Association, 1955), in which 38,000 persons 15 years and older were screened. This study was completed with a Darc Hemoglobinometer and cases showing a reading of under 12 grams per cent were reported as positive. The positive tests in this study represented 13.2 per cent of the total number screened.

In a hemoglobin screening survey completed on 3,203 persons, 15 years and older, undertaken by the Los Angeles City Health Department, 1952, (American Medical Association, 1955), 8.3 per cent were reported as positive. The copper sulphate method was used in the determination of hemoglobin in this survey.

The U. S. Public Health Service, in cooperation with the Department of Public Health and Hospitals of the City of Indianapolis, 1952, (American Medical Association, 1955), screened 5,711 adult negroes for low hemoglobin and found a total of 14.3 per cent positive. The screening levels used in

this survey was 12 grams per cent for females and 13 grams per cent for males.

The author's study does not compare favorably with the Vernon, Los Angeles County, 1952, hemoglobin screening survey. There were 572 employees of a printing establishment screened in this study and only eight persons screened positive or 1.4 per cent of the cases screened. This survey was completed using the copper sulphate method for the determination of hemoglobin, and was conducted by the employer and the employee's organization in the printing company. No group such as a health department, medical group, or other official health agency approved or sponsored the study, so it may be debatable whether this study should be used as a comparison.

Table II shows the distribution of positive males and females along with the mean hemoglobin for each interval.

There were 154 females, and 79 males who screened positive in the survey. The 154 positive females represented 16.3 per cent of the female population while the 79 positive males represented 13.6 per cent of the males screened.

These findings are much higher than the Boston Massachusetts Pilot Study number 1, 1950, (American Medical Association, 1955), in which only 3.6 per cent of the males screened positive and 2.3 per cent of the females were noted as positive. The screening levels used was 12.3 grams per cent for the males and 10.3 grams per cent for the females. The lower hemoglobin level used for the females would explain a portion of the percentage difference between the author's findings and this study.

In the Boston Massachusetts Pilot Study number 2, 1951, (American

TABLE II

DISTRIBUTION OF POSITIVE ADULTS; PERCENTAGE OF POSITIVE MALES
AND FEMALES BY INTERVAL; PREVIOUS HISTORY;
AND CASES UNDER MEDICATION *

AGE	NUMBER		NO. POSITIVE		% OF POSITIVE		PREVIOUS HISTORY		UNDER MEDICATION	
	Male	Female	M	F	M	F	M	F	M	F
21-24	19	20	1	2	5.3	10.0	0	1	0	0
25-28	11	43	1	7	9.0	16.3	0	5	0	3
29-32	31	49	4	8	12.9	16.3	0	3	0	1
33-36	42	72	5	12	11.9	16.6	0	6	0	1
37-40	62	98	7	26	11.3	26.5	0	16	0	3
41-44	69	108	14	21	20.3	19.4	0	8	0	3
45-48	59	105	7	20	11.9	19.0	0	9	0	3
49-52	64	90	7	15	10.9	16.6	1	5	0	2
53-56	53	105	10	10	18.9	9.5	1	4	0	1
57-60	52	81	7	9	13.5	11.1	0	5	0	3
61-64	34	55	3	8	8.8	14.5	0	1	0	1
65-68	34	49	3	8	8.8	16.3	1	2	0	2
69-72	21	35	6	6	28.6	17.1	2	1	1	0
73-76	13	22	0	1	0.0	4.5	0	0	0	0
77-80	10	5	1	1	10.0	20.0	0	1	0	0
81-84	7	7	3	0	42.8	0.0	0	0	0	0
TOTAL	579	946	79	154			5	67	1	23

* Note: This table is to be read in the following manner: there were 19 males in the age interval 21-24, there was one positive male in this age group, the percentage of positive males in this age group was 5.3, there were no cases with a previous history, and there were no cases under medication in this age group.

Medical Association, 1955), there were 8.3 per cent of the males with a hemoglobin level below 12.3 grams per cent and 1.8 per cent of the females with a hemoglobin level below 10.3 per cent. The mean ages of the adult populations screened in these two Boston Studies were not given. Perhaps with the considerably higher mean age of the Ellis County population there was a tendency for a higher positive rate due to a poor diet in the older group, or there may be a tendency for low hemoglobin to develop as a normal process of aging. The higher trend was shown with adults over 65 years of age.

Column 2 (Table II), lists a previous history of anemia if the person had ever had difficulty with anemia or a low hemoglobin. It is interesting to note that only five males of the total 79 positive males had a previous history of a low hemoglobin, while there were 67 females, or approximately one-half of the total 154 positive females, who had a previous history. A greater number of females rather than males probably showed a previous history because of the extra demands placed upon the female's body by the fetus during pregnancy, and the subsequent diagnosis of anemia by her physician.

This trend is further shown in column 3 (Table II), in which the number of persons receiving medications for anemia are listed. Only one male was receiving medications while there were 23 females who were receiving medications. This fact might indicate that many of the females suffering from anemia were diagnosed and receiving treatment because the condition was detected during pregnancy.

Since the percentage of positive males, 13.6 per cent, is almost as high as the percentage of positive females, 16.3 per cent, this may indicate

that there are many males in an adult population who would benefit from the treatment of anemia, but who are not normally diagnosed as being anemic.

There were 241 persons who had a previous history of a low hemoglobin but there were only 72 persons who had a previous history of a low hemoglobin and who screened positive in the survey. These figures indicate that there were 169 cases who had recovered from anemia under medical care, or who outgrew this condition. Many persons stated that they had anemia as a child.

There were 24 positive cases under medication at the time, and 72 positive cases with a previous history, which might benefit from treatment. These figures may indicate that although a patient has been diagnosed and is under treatment, he may not be receiving all of the benefits he might receive under intensified treatment. These figures may also indicate that after a person is diagnosed as anemic, receives some medication for his condition, and tends to feel progressively better, he may neglect to consult his physician regarding medications when his supply is exhausted, or he may discontinue medications because he feels that he no longer has anemia.

The survey showed that there were 161 positive tests with no history of a low hemoglobin. This figure represents 69.1 per cent of the total positive cases screened and is the figure with which the statistician is concerned since these are the new cases with no history of the condition for which they were screened.

Some of the reasons which the person felt was responsible for his low hemoglobin are as follows: three women stated that they always had extended menstrual cycles, seven persons had recently had surgery, one young female

had a miscarriage two weeks previously, and one older male had just had his teeth extracted and had been hemorrhaging excessively. The author had not planned to consider these cases in determining the positive means, but found that the levels did not significantly alter the mean of the males or females, so they were not deleted.

Figure 4 is a graph showing the percentage of positive cases in each age interval for the males and females. The largest percentage of positive males occurs in the age group 81-84 years, but there were only seven cases in this interval; an insufficient number of cases upon which to base a conclusion.

The largest percentage of positive cases for males was found in age group 69-72. The other two peaks for the male population was found in the age interval 53-56, and 41-44. This trend of positive males seems to indicate that the chances of a male suffering from low hemoglobin increases with age, especially after the age of seventy.

The percentage of positive females shows a somewhat different trend in that the percentage increases steadily from age 21 to about age 40, and then shows a decline to the age of 53, another increase to the age of 70, followed by a sharp decline to age 77.

This graph shows that the age interval in which a female is most likely to show a low hemoglobin is between the ages of 37-40. If she has not shown a low hemoglobin by the age of fifty, then she will be unlikely to have a low hemoglobin until she has passed the age of seventy or eighty. The high percentage of low hemoglobin shown in the higher age groups may be due to a lack of proper diet, or it may be a normal condition accompanying old age.

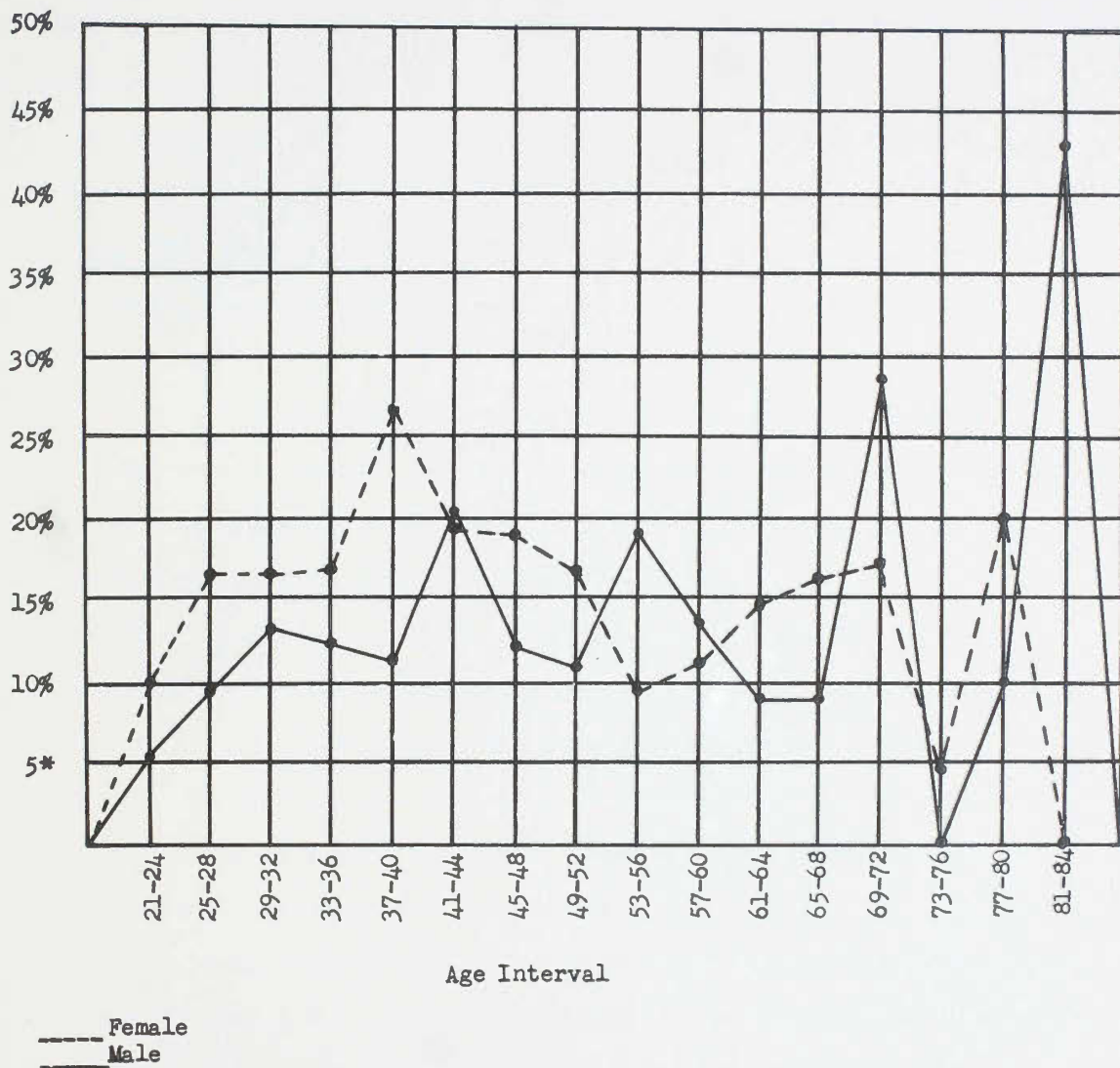


FIGURE 4
 PERCENTAGE DISTRIBUTION OF POSITIVE FEMALES
 AND MALES ACCORDING TO AGE

* Percentage frequency.

There is a striking similarity in the positive male and female trends shown in this graph from age 21 through age 50. The males however, show a four year lag in this positive trend, as compared with the females.

SUMMARY AND CONCLUSIONS

In October of 1959, 1525 hemoglobin screening tests using the cyanmethemoglobin method, were completed by the author on a sample of the adult population of Ellis and the surrounding counties of Kansas to determine the hemoglobin of males and females. The screening level, a value lower than 11 grams per cent for females and 12 grams per cent for males, was established and approved by the Ellis County Medical Association.

The hemoglobin screening survey showed a 15.3 per cent positive rate of the total number screened. Sixteen and three-tenths per cent of the total female population screened positive as compared with 13.6 per cent of the total males who screened positive.

There were only five males of the total 79 positive males who reported having a previous history of anemia, as opposed to 67 females of the total 154 positive females who reported that they had a history of anemia. One male was receiving medication while there were 23 females who were under treatment for anemia. There was a total of 161 positive tests with no history of a low hemoglobin.

This study seems to indicate that there are a sufficient number of adults who have a low hemoglobin to warrant a mass screening for this condition; that there are almost as many males who suffer from this condition as there are females; and that the cyanmethemoglobin method of hemoglobin determination, using the Leitz Colorimeter, can be efficiently and effectively carried out in a mass screening survey.

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